

STUDIES ON THE FREE AMINO ACIDS OF BLOOD AND SWEAT

BY

STANLEY WILLIAM HIER

B.S., College of the City of New York, 1939
M.S., University of Wisconsin, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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The individual free amino acids of blood and sweat have not been determined quantitatively previous to this study due to the limitations of the methods available. The development of microbiological assay methods for the amino acids has made possible studies of these important metabolites.

Microbiological Methods for the Determination of Free Amino Acids in Blood. — Media and methods are described for the determination of twelve amino acids microbiologically, in tungstic acid filtrates of plasma. The methods are shown to give values which are reproducible and valid. Tryptophane, leucine, isoleucine, and valine are determined with *Lactobacillus arabinosus*, arginine, phenylalanine, and tyrosine with *Lactobacillus casei*, histidine, lysine, and cystine with *Leuconostoc mesenteroides* P-60, and threonine and methionine with *Streptococcus fecalis*. Ten to twelve free amino acids in the plasma can be determined with a 50 cc. blood sample.

Studies on the Free Amino Acids in the Plasma of Human Subjects and Dogs under Normal and Abnormal Conditions. — Fasted blood samples from twenty-four male and nine female subjects were analyzed for ten free amino acids. No significant differences between the mean values for males and females were found. The normal values are as follows in mcg. per cc.: arginine 23.4 ± 6.2 , histidine 14.2 ± 1.8 , isoleucine 16.0 ± 3.1 , leucine 19.1 ± 3.4 , lysine 29.5 ± 4.2 , phenylalanine 13.8 ± 3.2 , threonine 20.2 ± 4.5 , tryptophane 10.8 ± 2.1 , tyrosine 14.8 ± 3.7 , and valine 28.3 ± 3.4 .

Similarly, values are reported for normal dog plasma as follows, in mcg. per cc.: arginine 32.7 ± 7.4 , histidine 12.4 ± 1.7 , isoleucine 13.1 ± 3.8 , leucine 20.9 ± 5.6 , lysine 24.2 ± 5.8 , phenylalanine 11.6 ± 2.9 , threonine 25.8 ± 7.3 , tryptophane 10.4 ± 1.7 , tyrosine 10.6 ± 2.4 , and valine 22.2 ± 5.3 . These do not differ significantly from the values for human subjects.

It is shown that the pattern of amino acids in the plasma proteins and muscle proteins is similar to that of the free amino acids in plasma. This may indicate an equilibrium relationship between these three systems.

Post-absorptive plasma from hospitalized patients suffering from liver cirrhosis, jaundice, nephritis, carcinoma, benign tumor, hyperthyroidism, hypertension, ulcerative colitis, splenomegaly, Hodgkin's disease, polycythemia, anorexia nervosa, multiple myeloma, rheumatoid arthritis, and amebic colitis were assayed for free amino acid content. The data show

some slight variations in the concentration of individual amino acids in the diseased conditions studies. However, the differences are neither very great nor consistent in all cases of the same disease so that they would not lend themselves to use as diagnostic indices. Studies of additional cases are indicated.

Plasma samples from pregnant women also fail to show any significant difference from the normal in free amino acid composition.

Two dogs were fasted for twenty-nine days but given free access to water. Blood samples were removed periodically for amino acid determinations. Both dogs showed a decrease in the level of arginine, histidine, phenylalanine, threonine, tryptophane, and tyrosine. Isoleucine, leucine, and valine rose, however. Lysine was variable. No explanation of these changes is offered except the possibility that changes in body protein reserves may influence the free amino acids.

Influence of Ingestion of Single Amino Acids on the Blood Level of Free Amino Acids. — Arginine, cystine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane, tyrosine, and valine were given singly by gavage to a number of dogs in the post-absorptive state. Successive blood samples were analyzed for amino acid changes. All of the amino acids given, except cystine, were found to rise significantly in the blood, usually reaching a maximum in sixty minutes and decreasing thereafter. After twenty-four hours the level was usually very close to normal. With methionine, however, even after twenty-four hours the level was still many times normal. Cystine rose only slightly, if at all. Methionine and histidine rose the most, while leucine, isoleucine, threonine, and valine were next, then tryptophane, phenylalanine, and tyrosine.

When the leucine level rose all of the other amino acids in the plasma fell except cystine, histidine, lysine, and tryptophane. With isoleucine feeding, arginine falls slightly, probably not significantly while the other amino acids show no marked change.

When dl-methionine is given the level in the blood goes up and remains high even after twenty-four hours, which is not found to be the case with the other amino acids. The body apparently has more difficulty in removing this amino acid than any of the others studied. At the same time, the levels of isoleucine, phenylalanine, tyrosine, and valine fall significantly.

Despite the fact that methionine rises and stays up so long, there is no increase in the cystine concentration. In fact, when cystine is given by mouth in the same amount as methionine, there is no increase in the cystine level of the blood.

However, when phenylalanine is given, as the phenylalanine blood level rises, the tyrosine level rises as well. When tyrosine is fed, it rises

without affecting phenylalanine. Arginine, histidine, threonine, valine, and tryptophane like tyrosine rise in the blood when fed, but do not affect any other amino acids.

The effect of methionine, leucine, and isoleucine in reducing the other amino acids, may be due to stimulation of a non-specific oxidase or deaminase or to peptide synthesis involving the amino acids which decrease.

Amino Acids of Human Sweat. — The same methods described for blood are applied to sweat. The normal values are found to be as follows in mcg. per cc.: arginine 135.8 ± 39.2 , histidine 80.2 ± 9.7 , isoleucine 22.7 ± 6.6 , leucine 26.9 ± 7.7 , lysine 22.6 ± 4.5 , phenylalanine 21.9 ± 6.3 , threonine 53.8 ± 16.4 , tryptophane 11.2 ± 3.3 , tyrosine 31.5 ± 9.5 , and valine 29.6 ± 7.5 .

The amino acid composition of the sweat is found to be the same regardless of whether the sample is taken from subjects in a fasting state or after eating. Furthermore, the level of the amino acids in sweat is independent of the variations in the blood. This is shown by the fact that when subjects ingested large amounts of amino acids, which raised the blood level considerably, there was no change in sweat amino acid content. It is also noted that the arginine and histidine content particularly, and the threonine and tyrosine content to a lesser extent, is higher in sweat than in blood plasma, while the other amino acids are present in about the same amounts. These observations indicate that the amino acids do not appear in sweat merely as a result of filtration from the blood plasma.

The amount of amino acids lost in the sweat is not strikingly significant in the economy of amino acids by the body. In our subjects the sweat contained about 0.44 mg. per cc. of the ten amino acids studied. Under average conditions of temperature and humidity, the amount of sweat is about 500 cc. per day. This would represent a loss of about 220 mg. per day of the ten amino acids. Even with excessive sweating in warm weather with active work when three liters of sweat may be formed, only 1.3 gm. of these amino acids would be lost.

VITA

Stanley William Hier was born in New York City, January 8, 1920. He received elementary and high school education in that city and a B.S. degree from the College of the City of New York in 1939. In 1940 he received the M.S. degree from the University of Wisconsin. He has been in charge of the Nutrition Laboratory, Research Laboratories, and Wilson Laboratories from 1940 to present.

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